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Controlled Crystallization of Acetazolamide from Aqueous Polymeric Solutions for Enhancing Dissolution Rate: Application of Statistical Moment Theory and Molecular Docking

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Presence of additives in crystallization process in a controlled manner can lead to different crystal morphologies which could have a favourable impact on drug dissolution rate**.** Four different hydrophilic polymers (methylcellulose, hydroxypropyl methylcellulose, polyvinyl alcohol, and carboxymethyl cellulose) were used for the controlled crystallization of acetazolamide (ACZ) by solvent evaporation technique. Crystal imperfections of ACZ occurred in the lattice of growing crystal when crystallized from aqueous polymeric solution and evaluated using both the traditional Full Width at Half Maximum (FWHM) (β) and statistical mean value of the XRD peak width (β′). Crystal imperfection has brought about significant improvement in the dissolution of newly produced acetazolamide crystals. ACZ crystal produced in presence of Hydroxypropyl methylcellulose (AHPMC) showed crystal imperfection to the maximum extent and also the greatest dissolution of the drug was noticed from AHPMC compared to other crystals. Statistical mean value of the peak width of XRD data as the error-free technique has been utilized successfully for estimating crystallite properties of acetazolamide crystallized from ethanol as solvent and aqueous polymeric solution as anti-solvent. Crystallite properties using traditional Full Width Half Maxima method and the error-free Statistical Moment Analysis were compared. This controlled crystallization technique could be utilized in the design and development of formulation for improved solubility and bioavailability of the drug.

Keywords: Carboxymethyl cellulose, Hydroxypropyl methylcellulose, Methylcellulose, Polyvinyl alcohol, Solubility improvement

Introduction

Crystal engineering is attempted for designing new solids with desired physicochemical properties.[1] Crystallization process is affected in presence of additives and it leads to different crystal morphologies which have a significant impact on drug release properties besides tablet compaction process.[2] Alternative crystallization techniques are applied in crystal engineering for producing pharmaceutical materials of improved properties. Many traditional methods can be used to create crystalline drug products, such as solvent vaporization, pH adjustment, application of heat, vapour diffusion, growth in the presence of additives (surface active agents/polymers), etc.[3–6] To increase the solubility and rate of breakdown of the active ingredients, crystallisation in the presence of polymeric solution is one of these that is being extensively researched.[7–9] The presence of the right polymer(s) in the vehicle in the desired amount may help to keep the drug supersaturated. Polymers alter the viscosity of the medium and have an impact on the nucleation phase during crystal formation at the exact same time, which causes a change in polymorphic shapes or crystal habits. Acetazolamide inhibits carbonic a hydrase and reduces the secretion of aqueous humor after systemic administration as tablets, capsules, and intravenous solution for the control of glaucoma. Metabolic acidosis, diuresis, anorexia, nausea are the associated systemic adverse effects after oral use of acetazolamide. Poor aqueous solubility and low tissue permeability are the reasons for limiting the ocular bioavailability of acetazolamide.[10] Topical administration of acetazolamide could decrease these adverse effects significantly. Crystallisation of acetazolamide in presence of hydrophilic polymer in a controlled manner could improve the solubility of the drug due to the impact on the crystal habit. An aqueous solution of hydroxypropyl methylcellulose (HPMC),

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methylcellulose (MC), carboxymethyl cellulose (CMC), and polyvinyl alcohol (PVA) were used as non-solvent in the crystallization process of acetazolamide in ethanol as solvent. Crystals were evaluated for physicochemical and in vitro dissolution properties. The Full Width at Half Maximum (FWHM) is the difference between two values (x-axis) at which y-value is equal to half of its maximum value in a particular distribution if the function is symmetric (Fig. 1). However, XRD often demonstrates asymmetric peaks and causes error in evaluating FWHM.[11]

Moment analysis is an important statistical tool used for quantification of parameters in Biopharmaceutics and Pharmacokinetics. The non-compartmental analysis in pharmacokinetics is based upon statistical moment theory which involves the collection of experimental observed data after administration of a single dose of drug. One important pharmacokinetic parameter, Mean Residence Time (MRT) can be estimated using statistical moment theory without the complicated nonlinear regression approach of compartmental analysis which involves application of mathematical equations based upon a set of assumptions (error based having sum of residual squares).[12–14] Practically AUC and AUMC can be estimated from the respective graphs by the trapezoidal rule. This method involves dividing the curve by a series of vertical lines into a number of trapezoids, evaluating separately the area of each trapezoid and adding them together.

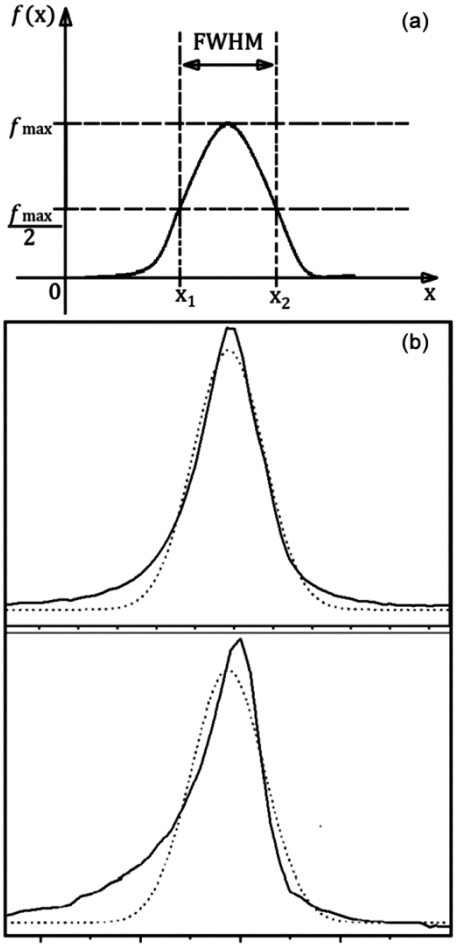

Fig. 1 — (a) Full Width at Half Maximum (FWHM): the difference between two values (x-axis) at half of its maximum y-value in a symmetric function; (b) Asymmetric peak causes error in evaluating FWHM

AUC = area under the plasma concentration (C) – time (t) curve, AUMC = area under the first moment curve (area under the $C.tvs.\ t$ curve), MRT = Mean Residence Time = statistical mean value in time

$\text{AUC}=\int_0^t C.dt$ and,

$\text{AUMC}=\int_0^t C.t.dt$

$\text{MRT}=\frac{AUMC}{AUC}$

Present study was undertaken for adopting an alternative error-free technique to estimate the mean value of the peak width of XRD data using statistical moment analysis (mean peak width = AUMC/AUC) for assessing crystallite properties of acetazolamide after crystallizing from aqueous polymeric solution. This type of error-free procedure for evaluating crystal imperfection parameters related with the dissolution improvement of acetazolamide has not been reported earlier.

Materials and Methods

Materials

Acetazolamide (ACZ) was obtained from Celogen Pharma Pvt Ltd, Navi Mumbai, India, as a gift sample. Hydroxypropyl methylcellulose (HPMC), Molecular Weight: 1261.4 gm/Mol, Carboxymethylcellulose (CMC) Molecular Weight: 262.19 gm/Mol, Methylcellulose (MC) Molecular Weight: 454.5 gm/Mol, and Polyvinyl alcohol (PVA) Molecular Weight: 85000–124000 gm/Mol were purchased from Merck Private Limited, Mumbai, India.

Methods

Pure acetazolamide (0.5 gm) was dissolved in 80 mlethyl alcohol with constant stirring of 100 rpm at 50°C in a one-liter crystallization vessel (Table 1).[15] Then 400 ml polymer solution (0.5%, w/w) was slowly added at a rate of 3.3 mL/min[16] with

Table 1 — Acetazolamide crystallization from aqueous polymeric solution

Formulation code	Acetazolamide (gm)	Polymer (0.5%)	%Yield (Mean ± SD)
ACMC	0.5	CMC	89 ± 1.6
APVA	0.5	PVA	84 ± 1.4
AMC	0.5	MC	81 ± 0.9
AHPMC	0.5	HPMC	80 ± 1.6

simultaneous cooling of 50 to 10℃ (22℃ per minute). The formed crystals were rinsed properly to remove excess amount of polymer adhered in the formed crystals and left for characterization. The percent yield of crystals was evaluated and their properties were assessed.

Light Microscopy

The surface structure, shape, and texture of the crystallized acetazolamide products if any were visualized by an optical microscope. A trace amount of the sample was put on a glass slide and observed under the microscope under visible light at room temperature.

Scanning Electron Microscopy (SEM)

For examining the three-dimensional shape and texture of acetazolamide crystal and the formulated crystals photomicroscopy (ESEM-FEI Quanta-250) was used.[17] The crystalline morphology was visualized by introducing a high beam of electrons and accelerated voltage of 5/15 Kv.

ATR-FTIR Spectroscopy

ATR-FTIR spectroscopy was used for analyzing chemical bonding interactions of the crystal samples and the pure drug crystals using FTIR spectrometer (FT/IR-4600, Jasco).[18] Samples were placed in contact with the zinc selenite surface as the internal reflection elements applying integrated pressure device.

Powder X-Ray Diffraction (PXRD)

Using an X-ray diffractometer, the XRD patterns of pure acetazolamide and the crystals were examined (Rigaku ultimate PXDL software). The corresponding voltage and amperage were 40 kV and 15 mA. Cu, K-alpha anode material (1.5406) was used to measure the diffraction at a rate of 1 per minute between 5 and 50.[19]

In-vitro dissolution study

USP paddle-type dissolution apparatus was used in in-vitro dissolution.[20] Biologically relevant fluid (buffered at pH 7.4) was used as dissolution medium (paddles were rotated at 50 rpm; 37 ± 0.5°C). The collected samples were characterized under UV-Visible spectroscopy (Jasco-4100). Drug release was performed three times and the mean value was noted.[21]

In-silico molecular docking study

Auto Dock Vina 1.1.2 program was used for evaluating binding between drug and polymer. Interaction between acetazolamide and polymer was pre-calculated using this program. The output of drug-polymer interaction energy was recorded. The more negative score is the indication of the better binding.

Results and Discussion

An anti-solvent strategy was used to produce ACZ crystals. The anti-solvent, a liquid solution of polymer, was used while the primary solvent, ethanol, was used to solubilize ACZ. To encourage controlled crystallisation, polymer prolonged the supersaturation process and raised the medium's viscosity.[15] The packing or structure of molecules in crystal/atoms in the pure ACZ crystal was collected from the source: (Pubchemis presented in Fig. 2).

Light Microscopy

Light microscopy study revealed the changes in crystal morphology of seen in Fig. 3. The particle size has been reduced and the change in shape, texture and surface structure of the crystallized products were clearly observed in the images. The existence of polymer might have inhibited the regular crystallization method and affected the size, shape, texture, and surface structure of the produced acetazolamide crystals.

Scanning Electron microscopy (SEM)

The surface texture and shape of acetazolamide crystal and the formulated crystals photomicrographs are presented in Fig. 4. Acetazolamide crystals are seen as the geometric brick-shaped form in the micrograph images.[16] The distinct crystalline shape has been lost in the micrograph image of crystal samples. Probably polymer in the desired quantity in-vehicle assisted in maintaining supersaturation level of drug and hampered the traditional crystal geometry.

ATR-FTIR Spectroscopy

Infrared spectra of acetazolamide and different crystals were carried out and presented in Fig. 5. Characteristic absorption bands of acetazolamide

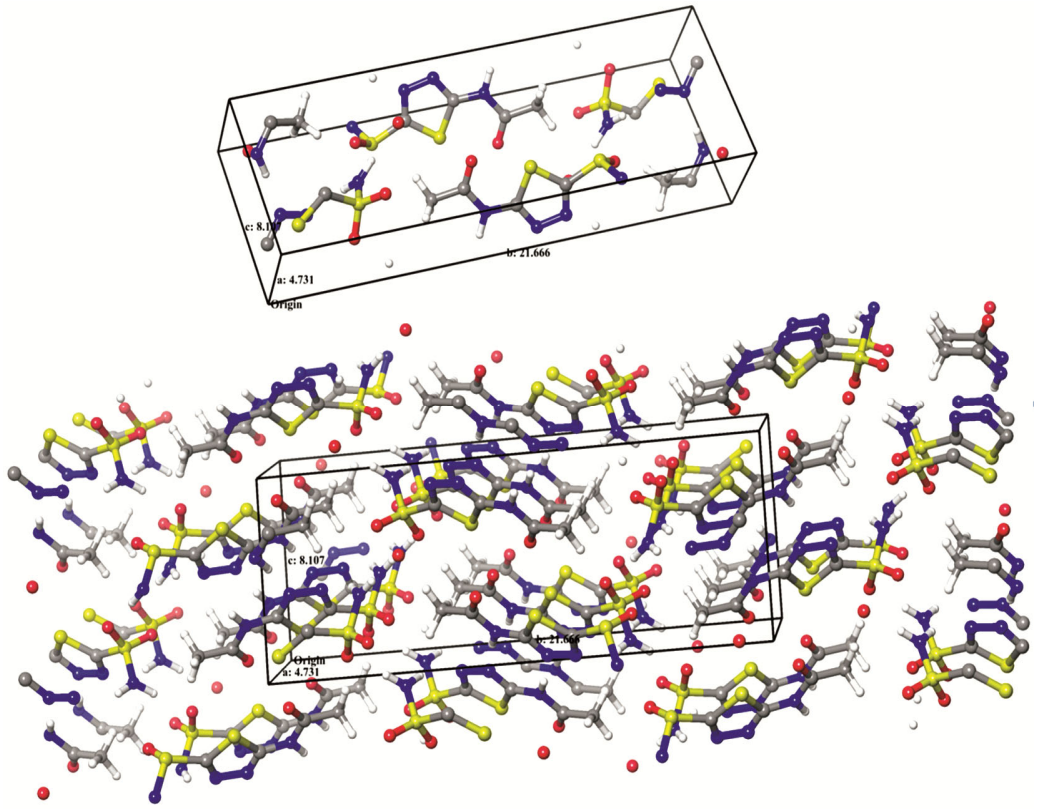

Fig. 2 — Pure acetazolamide crystal packing arrangement pattern or packing of molecules/atoms (*source: Pubchem*)

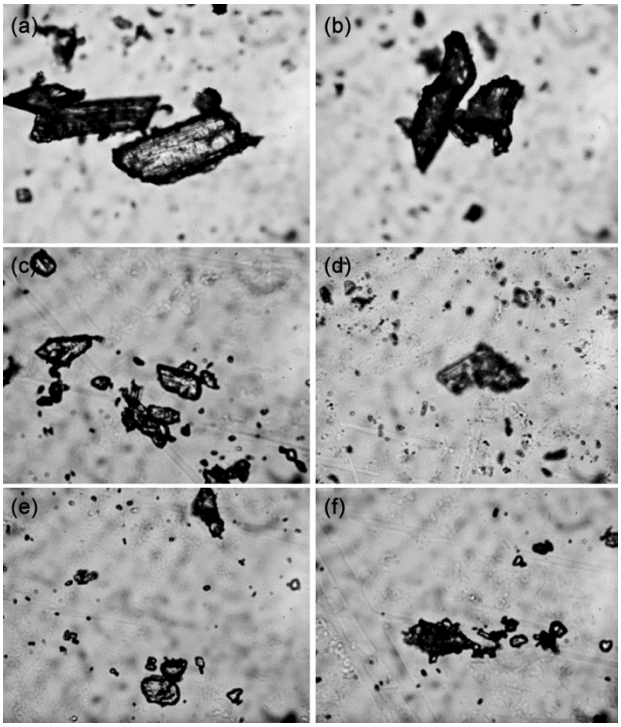

Fig. 3 — Light microscopic image of the ACZ crystals (a and b: ACZ, c: ACMC, d: APVA, e: AMC, f: AHPMC)(Magnification 100 X)

were found at1671 (C = O stretch), 1536 (N-H), and 1110–1310 (S = O) cm^{-1} were present (Form A acetazolamide).[22] The reduction in the intensity of the transmittance was due to progressive amorphization of the drug. Acetazolamide absorption bands at 1671 and 1536 cm^{-1} were distinctly appeared in the crystal samples.[23] This result suggested that the crystal nature was slightly changed due to the polymeric effect. A significant change in the band position and new peak appearance were not found during the crystallization process of acetazolamide.

Powder X-Ray Diffraction (PXRD)

XRD of pure acetazolamide and crystals has been displayed in Fig. 6. The XRD of acetazolamide showed high-intensity reflections with typical peaks at 9.9, 17, 20, 23, 25, and 29° 2θ angle.[21] In the crystal form of acetazolamide all the 2θ angles are showing low intensity due to the interfering effect of polymer. FWHM, particle size, strain in lattice, and dislocation density were measured using the following traditional method.

The particle size and strain in lattice were determined from the following equation.[24–26]

$$\varepsilon = \beta/4tan\theta \quad \ldots (1)$$

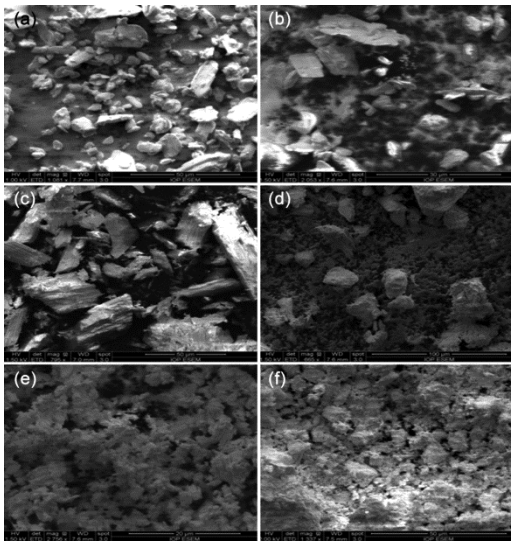

Fig. 4 — Photomicrograph images after scanning electron microscopy of (a) ACZ, (b) ACMC, (c) APVA, (d) AMC, and (e) AHPMC

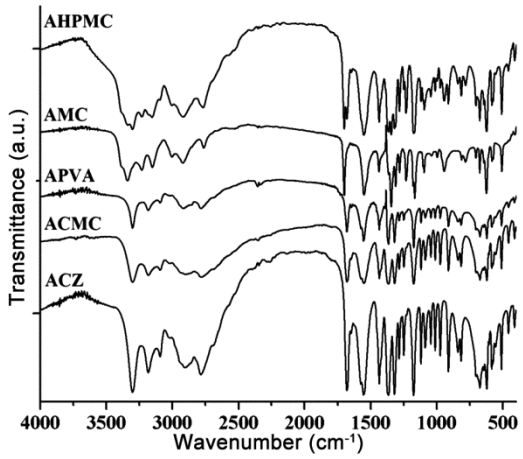

Fig. 5 — FTIR spectra of ACZ crystals (ACZ, ACMC, APVA, AMC, and AHPMC)

Crystallite size was evaluated as per equation below:

$$D = 0.9\lambda/\beta cos\theta \quad \ldots (2)$$

where, ε = Strain, β = Full-Width Half Maxima (FWHM)

D = Crystallite size, λ = Wavelength

The dislocation density (δ) representing the amount of imperfections of the prepared crystal is defined as the length of dislocation lines per unit volume of the crystal and is evaluated using Eq. (3)

$$\delta = 1/D^2 \quad \ldots (3)$$

Area under the XRD peak (AUC), area under the first moment XRD peak (AUMC), and the mean value

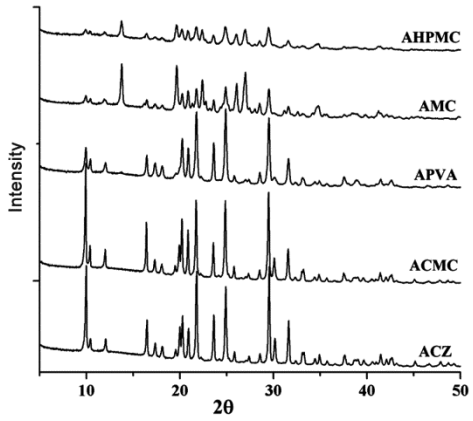

Fig. 6 — X-ray diffraction pattern of the crystals (ACZ, ACMC, APVA, AMC, and AHPMC)

of the peak width were determined using statistical moment analysis (mean x-axis value evaluated by AUMC/AUC). In the present research error-free particle size and strain in lattice were determined using the mean value of the peak width as β′ (analogous to FWHM) from the Debye-Scherrer's equation and tabulated in Table 2.

The Crystallite size of acetazolamide has been decreased[27] in presence of polymer. β and β′along with crystal strain increased when crystallized in presence of polymer. Dislocation density has also been increased indicating the crystal imperfection of acetazolamide crystallized from aqueous polymeric solution.

Drug Release Study

In-vitro dissolution profiles of all the formulated crystals with pure ACZ have been depicted in Fig. 7. Drug release has been enhanced significantly (85 to 94%) in 120 min in all the crystal formulations compared to the pure drug (73%).[28] The AHPMC enhanced the release of acetazolamide to the highest extent (98%) compared to the other crystals due to the highest crystal imperfection. Drug release rate has been increased as a function of the degree of decreased crystallite size with the increased crystal strain and dislocation density of acetazolamide crystal in presence of polymer. The extent drug release was noticed as ACZ < ACMC < APVA < AMC < AHPMC.

***In-silico* Molecular Docking**

The drug Acetazolamide formed different complexes through Hydrogen bonds and hydrophobic bonds with all four polymers (Fig. 8). The different formed crystals showed a change in binding affinities/energies ACMC: −1 Kcal/mol, APVA: −2.2 Kcal/mol, AMC: −2.4 Kcal/mol and AHPMC: −2.5

Table 2 — Crystal imperfection parameters of acetazolamide crystallized from aqueous polymeric solution (mean ± SD): β vs. β'

Sample	Full Width Half Maxima method			Statistical Moment Analysis			Dislocation density (m^{-2}) $\times10^{-3}$	% Drug release at t_{120}	Docking energy (Kcal/mol)
	D (nm)	β	Strain $\times10^{-3}$	D (nm)	β'	Strain $\times10^{-3}$			
ACZ	52.1 ± 5.4	0.164 ± 0.018	7.9 ± 0.39	48.5 ± 4.2	0.172 ± 0.015	3.75 ± 4.25	76.66 ± 28.50	72.95 ± 0.72	—
ACMC	47.3 ± 2.1	0.180 ± 0.010	10.2 ± 0.22	35.0 ± 3.4	0.239 ± 0.007	7.23 ± 1.97	82.32 ± 24.95	84.06 ± 1.73	−1
APVA	35.4 ± 1.6	0.240 ± 0.009	13.5 ± 1.60	22.4 ± 2.5	0.378 ± 0.008	18.13 ± 1.85	82.48 ± 24.88	89.64 ± 2.22	−2.2
AMC	26.5 ± 3.5	0.323 ± 0.050	15.6 ± 8.45	22.3 ± 3.8	0.379 ± 0.061	18.24 ± 7.95	68.21 ± 21.69	94.31 ± 1.52	−2.4
AHPMC	16.4 ± 3.3	0.649 ± 0.107	31.9 ± 3.60	16.6 ± 1.6	0.514 ± 0.100	30.80 ± 4.01	82.33 ± 24.77	96.97 ± 1.73	−2.5

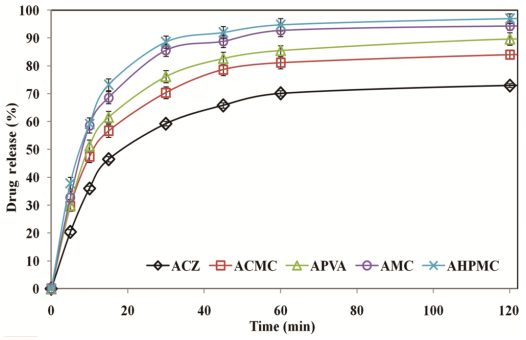

Fig. 7 — In-vitro dissolution profile of acetazolamide crystals

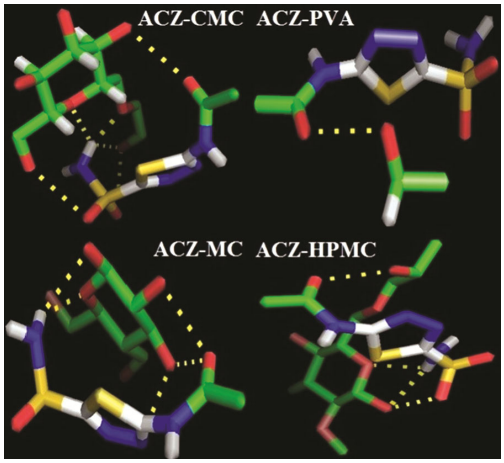

Fig. 8 — Molecular docking interaction

Kcal/mol. The higher negative binding energy values indicate stable interactions than that of lower negative values. The highest negative binding energy value of AHPMC crystal product indicates stable interactions to the highest extent due to the effect of HPMC as supported by the above characterization compared to the least binding energy due to CMC (ACMC).[29–31]

Conclusions

Controlled crystallization technique has been utilized for producing acetazolamide crystal for enhancement of dissolution rate of the drug. In the present study error-free particle size and strain in lattice were determined using statistical moment analysis after estimating the mean value of the peak width of XRD data. The estimated area under the XRD peak (AUC), the area under the first moment XRD peak (AUMC), and the mean value of the peak width (mean x-axis value evaluated by AUMC/AUC) were utilized for the determination of crystal imperfection parameters. This crystal imperfection resulted in significant improvement of the dissolution of acetazolamide of all the crystals. The estimated maximum value (β′) of AHPMC indicated the crystal imperfection of ACZ to the highest level when crystallized in existence of hydroxypropyl methylcellulose and AHPMC brought about the highest release of ACZ compared to other crystals. Likewise, the outcome was maintained by x-ray diffraction and SEM studies. This new technique could help in estimating error-free crystal properties and may be utilized in formulation development for improved solubility as well as bioavailability.

Conflict of interest

We declare the there is no conflict of interest.

Acknowledgements

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